Heparin Extraction of Nuclear Envelopes

(Rout Lab, 1998)

Purpose: to extract proteins from the nuclear envelope using heparin

1. In a TLA-45 centrifuge tube, add 25 - 50 µl of a nuclear envelope preparation

2. EXTRACTION STEP:
Add 4-9 volumes of the extraction solution and vortex 30 sec. to mix

Extraction solution (for 10 mL):
- 1 mL of 100 mM Bis-Tris/$MgCl_2$, pH 6.5 (Cf = 10 mM)
- 1 mL of 100 mg/mL heparin (Cf = 10 mg/mL)
- 1:500 dilution of solution P (a standard protease inhibitor mix)

3. Incubate mixture on ice for 1 hour to O/N.

4. Spin in TLA-45 rotor in TL-100 centrifuge for 20 min. at 40K, 4˚C

5. Transfer supernatant to a fresh microcentrifuge tube, and add 500 µl of H_2O to the TLA-45 tube containing the pellet.

6. PRECIPITATION STEP: To both the supernatant and the pellet, add:
- 500 µl H_2O
- 200 µl 0.15% NaDOC (a protein carrier to pellet the proteins in the solution)
- 100 µl 72% TCA (convertes deoxycholate to deoxycholic acid, and precipitating the proteins into a flocculent white pellet)

Mix by inversion and incubate on ice for 1 hour.

7. Spin at 15K for 20 min. at 4˚C

8. Aspirate off supernatant, and resuspend pellet in 0.5 mL 90% acetone. Acetone solubilizes the deoxycholate and leaves a protein-enriched pellet.

9. Incubate at -80˚C for 20-30 min.

10. Spin at 15K for 20 min. at 4˚C

11. Aspirate supernatant, air-dry pellet 5 min.

12. Add 5 µl solution A (0.5 M Tris, 5% SDS) and 5 µl solution B; heat at 95˚C for 10 minutes.

13. Run the whole sample on a 5% - 20% gradient SDS-acrylamide gel.